

**REMARKS UNDER 37 CFR § 1.111**

**FORMAL MATTERS**

Claims 1-45 are pending after entry of the amendments set forth herein.

Claims 1-45 were examined. Claims 34-44 were withdrawn from consideration. Claims 1-33 and 45 were rejected.

Claims 1, 32 and 45 are amended. Support for these amendments is found throughout the specification, as well as in the claims as originally filed and/or previously presented.

Applicants respectfully request reconsideration of the application in view of the amendments and remarks made herein.

No new matter has been added.

**INTERVIEW SUMMARY**

Applicants wish to extend their gratitude to Examiner Johannsen for the telephonic interview conducted with the undersigned on October 21, 2004, as well as for the subsequent follow-up calls. The rejections of the claims under §112, ¶2 and §§102(a) and (e) were discussed during the October 21, 2004 interview. Proposed amendments similar to those presented here were also discussed. The Examiner indicated that the proposed amendments would be entered, and would not raise new issues.

The Examiner agreed that the amendments presented herein render the rejection of the claims under §112, ¶2 moot.

As to the rejections under §§102(a) and (e) based on Cronin, the Examiner stated that it was not clear that the total ionic salt concentration in Cronin was greater than 0.7M, and thus outside the scope of the proposed claims and as per the claims as amended here. The Examiner specifically pointed to the language in Cronin that:

Labeled, fragmented **PCR product** (range=20 to 60 bases, average length=40 bases) **was diluted 10 to 25 fold into 5X SSPE (750 mM NaCl, 50 mM NaPhosphate, 5 mM EDTA, pH 7.4) and 1 mM cetyltrimethylammonium bromide (CTAB, Sigma) and used directly in hybridizations.**

(Cronin, column 47, lines 46-51, emphasis added).

However, as applicants clarified in a follow-up telephone call to the Examiner, and as discussed in detail below, Cronin requires that the **PCR product** be **diluted into** 5XSSPE. The *PCR product* is diluted 10-25 fold, not the opposite where the 5XSSPE is diluted 10-25 fold as suggested by the Office.

In support of this, applicants note that Cronin clearly states in the next paragraph that

**Targets were hybridized separately in 1-3 ml of 5X SSPE with 10 mM CTAB at 30°C. for 30 minutes.**

(Cronin, column 47, lines 56-68).

We now turn to each of the rejections in detail.

### **REJECTIONS UNDER §112, ¶2**

The claims were variously rejected under §112, ¶2. Applicants respectfully traverse these rejections are applied and as they may be applied to the pending claims. Each of these rejections is addressed below.

### **Claims 1-33 and 45 -- “Specific duplexes” and “specific nucleic acid duplexes”**

This rejection is rendered moot by deleting the objected language from the claims.

**Claims 1-33 - “Whereby differences in the extent of specific duplex formation discriminate . . . at the level of a single nucleotide difference”**

Applicants respectfully submit that this language is meant to refer to detection at the level of a single nucleotide difference between matched and mismatched duplexes. Claim 1 is amended to provide further clarification, rendering this rejection moot.

**Claim 29 – “wherein . . . less than about 0.7M total ionic salt concentration”**

Claim 29 is canceled, rendering this rejection moot. Amended claims 1 and 45 recite “less than 0.7M total ionic salt concentration”

**Claims 32-33 “adding salt . . . reaction comprises greater than 0.7M total ionic salt concentration”**

The Examiner kindly explained this rejection during the interview. Amendments to claim 32 are to further clarify that salt is added *after* duplex formation in claim 1.

**Conclusion**

Withdrawal of all rejections of the claims under §112, ¶2 is respectfully requested.

**REJECTIONS UNDER §§102(A) AND (E) – CRONIN**

Claims 1-33 and 45 were rejected under §102(e) and §102(a) as being anticipated by Cronin (US 6,027,880). This rejection is respectfully traversed as applied and as it may be applied to the pending claim.

Claim 1 and 45 as amended require that the total ionic salt concentration of the hybridization reaction is less than 0.7M.

**Cronin does not disclose use of CTAB in a hybridization reaction having a total ionic salt concentration of less than 0.7M**

The only disclosure in Cronin relating to CTAB states that it is to be used in used in a hybridization reaction with **0.75M NaCl**:

Labeled, fragmented **PCR product** (range=20 to 60 bases, average length=40 bases) **was diluted 10 to 25 fold into 5X SSPE (750 mM NaCl, 50 mM NaPhosphate, 5 mM EDTA, pH 7.4) and 1 mM cetyltrimethylammonium bromide (CTAB, Sigma) and used directly in hybridizations.**

Target was diluted into hybridization solution (10-40 nM final concentration, depending on PCR yield) and hybridization was carried out by agitating the DNA probe array in a 25 mm tissue culture dish placed in a temperature controlled shaker/incubator. **Targets were hybridized separately in 1-3 ml of 5X SSPE with 10 mM CTAB at 30°C. for 30 minutes.** The arrays were washed briefly (1-5 minutes) at 25.degree. C.-30°C. with 5X SSPE and 0.01% SDS prior to imaging. A preliminary series of experiments established that 10 nM target begins to approach saturation of complementary probe hybridization sites within 30 minutes.

Cronin, column 47, lines 46 - 62.

As discussed above, during the interview the Examiner stated that it was not clear that the total ionic salt concentration in Cronin was greater than 0.7M, and thus outside the scope of the amended claims. The Examiner specifically pointed to the language in Cronin that:

Labeled, fragmented **PCR product** (range=20 to 60 bases, average length=40 bases) **was diluted 10 to 25 fold into 5X SSPE (750 mM NaCl, 50 mM NaPhosphate, 5 mM EDTA, pH 7.4) and 1 mM cetyltrimethylammonium bromide (CTAB, Sigma) and used directly in hybridizations.**

(Cronin, column 47, lines 46-51, emphasis added).

However, as discussed above, Cronin requires that the **PCR product** be **diluted into** 5XSSPE. The **PCR product** is diluted 10-25 fold, not the opposite. The 5XSSPE is not diluted 10-25 fold as suggested by the Office.

In support of this, applicants note that Cronin clearly states in the next paragraph that

**Targets were hybridized separately in 1-3 ml of 5X SSPE with 10 mM CTAB at 30°C. for 30 minutes.**

(Cronin, column 47, lines 56-68).

**CTAB will not work as a specific association enhancer where the ionic salt concentration is 0.7M or greater -- it removes CTAB from the complex entirely, defeating the activity of CTAB as a specific association enhancer**

Furthermore, and as previously presented, Applicants have found that use of CTAB at a total ionic salt concentration greater than 0.7 M does not provide an environment in which CTAB can act as a specific association enhancer as required by the claimed methods. In fact, applicants have found that at this total ionic salt concentration, CTAB is not associated with any DNA duplex.

The specification shows that increasing the "ionic salt concentration [*e.g.*, NaCl concentration] to at least about 0.7M" "**disrupt[s] the association of the specific association enhancer and nucleic acid duplex.**" Specification p. 22, lines 18 – 21 (emphasis added).

The specification further states that "[w]here the specific association enhancer is a cationic detergent, such as CTAB or a CTAB-like cationic detergent, the invention further presents, in another aspect, methods **to remove the specific association enhancer** from the hybridization reaction," by increasing the salt concentration "until said hybridization reaction comprises greater than 0.7M total ionic salt concentration." Specification, lines 18 – 20.

**Conclusion**

In view of the above, it is evident that Cronin does not teach the method of the invention. Instead, and at best, Cronin teaches hybridization conditions that would not result in the claimed method, and in fact result in the dissociation of CTAB from the nucleic acid duplex – defeating the entire purpose of the claimed invention.

Therefore, applicants respectfully submit that Cronin does not anticipate the claims under either §102(a) or §102(e). Withdrawal of these rejections is respectfully requested.

**CONCLUSION**

Applicant submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

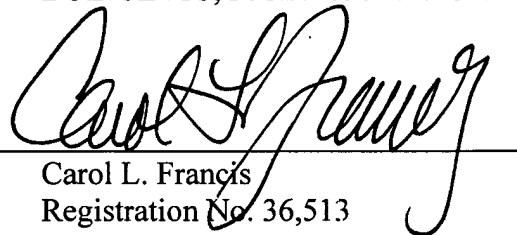
The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number STAN-202

Respectfully submitted,  
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Nov 12, 2004

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